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Poster Presentations

Lucie Steiningerova, J Clin Exp Pathol 2018, Volume 8
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Homologues of F420H2-dependent reductases from the biosynthesis of Lincomycin, Hormaomycin, and Pyrrolobenzodiazepines have different reaction specificity

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In contrast to taxonomically ubiquitous flavin-dependent oxidoreductases, deazaflavin F420/F420H2 dependent oxidoreductases are limited to the phyla *Euryarchaeota* and *Actinobacteria*. Within *Euryarchaeota*, these enzymes play a key role in the central catabolic CO2 reducing and methylotrophic pathways. Within *Actinobacteria*, their function is less explored and possibly more diverse, including participation in the biosynthesis of secondary metabolites. However, the only functionally elucidated example is the reduction of a double bond of dehydrooxytetracycline during the final step of tetracycline antibiotic biosynthesis. Homologues of F420/F420H2 dependent oxidoreductases are encoded also within the biosynthetic gene clusters of three groups of structurally and functionally distinct natural products, which all incorporate an unusual 4-alkyl-L-proline into their structures: (1) lincomycin, a clinically used lincosamide antibiotic, (2) pyrrolobenzodiazepines with antitumor properties, and (3) hormaomycin, a signal molecule involved in the quorum-sensing system. In this work, we prepared five recombinant homologues of F420H2 dependent enzymes putatively involved in the biosynthesis of 4-alkyl-L-proline of the above-mentioned metabolites. Further, we isolated the substrates and a deazaflavin cofactor from the culture broths of *streptomycetes* and *mycobacteria* and we set up in vitro enzymatic assays, which we monitored by LC-MS. We revealed that the reductase from the biosynthesis of lincomycin catalyzed an unusual reduction of two conjugated double bonds, while the reductases from pyrrolobenzodiazepines and hormaomycin biosynthesis converted the same substrate into a product, in which one of the double bonds remained intact. These results comply and fit within the biosynthetic pathways of the relevant metabolites and they represent the first example of homologues of F420H2 dependent reductases exhibiting different reaction specificity.

Biography

Lucie Steiningerova has been a member of the Laboratory for Biology of Secondary Metabolism since her bachelor degree. Currently, she is a student of the third year of Doctoral studying program Microbiology at the Charles University in Prague. The aim of her PhD thesis concerns biosynthesis of secondary metabolites in *Streptomyces*-lincosamide antibiotics, anticancer pyrrolobenzodiazepines, and hormaomycin.

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Magdalena Pavlikova, J Clin Exp Pathol 2018, Volume 8
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The characterization of oral micro biome in periodontally healthy people

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Periodontitis is a serious inflammatory disease affecting supporting structures of teeth (periodontium). Bacteria play an essential role in disease development; they accumulate in the subgingival space and form biofilms. Depending on the velocity of progression of the tissue destruction we distinguish the aggressive and chronic type of periodontitis. The probability of development of chronic periodontitis disease increases with the age of the patient. Thanks to modern sequencing methods, the oral taxa present in patients with periodontitis are generally well described. The characterization of the oral microbiome associated with periodontal health, however, could be radically improved. According to our preliminary results we define the onset of chronic periodontitis as a continuous process with the gradual colonization of mouth pathogenic bacteria. The aim of our project is a characterization of oral microbiome in typically healthy persons on a group of 60-100 young people, using the Illumina MiSeq sequencing method. We suppose that in a group of these very young people there is the biggest probability to obtain bacterial profiles without any periodontal changes. A better definition of oral microbiome associated with periodontal health will subsequently enable the use of sequencing methods for early diagnosis of patients with periodontitis.

Biography

Magdalena Pavlikova has been a member of the Laboratory for Biology of Secondary Metabolism since her bachelor degree. Currently, she is a student of the third year of Doctoral studying program Microbiology at the Charles University in Prague.

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Probiotics with *Lactobacilli* origin differently act on the growth of hospital-acquired multidrug-resistant *Klebsiella Pneumoniae*

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The positive effects of probiotic *lactobacilli* on human organism are known. Previously we suggested the use of Verhulst's equation (VE) for the evaluation of the growth of gut isolates from the patients with Familial Mediterranean fever disease. We used VE for the characterization of the growth of hospital-acquired multidrug-resistant *Klebsiella pneumoniae* strain in current investigations. The differences in the duration of the growth preparatory and logarithmic phase, as well as in the specific growth rate of the pathogen were detected under the influence of putative probiotic *lactobacilli* strains: *L. rhamnosus* str. Vahe, *L. rhamnosus* str. ASAP, *L. rhamnosus* str. *Lacto-G*, *L. plantarum* str. ZPZ and *L. delbrueckii* str. IAHAHI. The strain-specific effects of *lactobacilli* on pathogen's growth show a possibility to use VE in the design of pharmacokinetics of probiotics. This study was supported by the International scientific Technical Center (A-2134).

Biography

Lilit Malkhasyan received her MSc from the Armenian National Agrarian University (specialty:children and functional nourishment technology). She is an active member of the International Association for Human and Animals Health Improvement, Yerevan, Armenia. She is the author of three scientific publications.

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Dual labeling of *Pseudomonas Putida* ND6 with fluorescence proteins for exploring the conjugal transfer of pND6-1 and pND6-2 plasmid

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Dual labeling of *Pseudomonas putida* ND6 with fluorescence proteins for exploring conjugal transfer of pND6-1 and pND6-2 Plasmid: Gram-negative *Pseudomonas putida* ND6 possess two large plasmids pND6-1 and pND6-2. The former one which carries the genes encoded for naphthalene degradation in the catechol-meta-cleavage pathway belongs to the IncP-7 conjugative plasmid. Several genes involved in the Type IVB Secretion System are located in the later plasmid. In order to well-understand the characteristics of these two plasmids during conjugation, pND6-1 and pND6-2 were labeled with red fluorescent protein gene (dsred) and green fluorescent protein gene (gfp) respectively by homologous recombination via biparental mating. In view of the narrow host range of the IncP-7 plasmid, Poprl promoter (located before the oprl gene) from *Pseudomonas putida* ND6 was attached to dsred and gfp and inserted into the non-functional region of plasmid together to avoid affecting the expression of functional genes on the plasmid. Both red and green fluorescent proteins were co-expressed in the isolated conjugon GROND6 (pND6-1::dsred, pND6-2::gfp). Furthermore, the results suggested that Poprl promoter could better improve the red fluorescent expression when compared with the green fluorescent protein in *P. putida* ND6. The dual-labeled GROND6 with red and green fluorescent proteins was subsequently tested its conjugation transfer by mating experiment with *P. putida* KT2440 as the recipient. The screened transconjugant KT2440RG exhibited both red and green fluorescence under fluorescence microscopy, indicating that the constructed dual-fluorescent-labeled strain GROND6 (pND6-1::dsred, pND6-2::gfp) can be used to in situ detect the transfer of two mobile plasmids in ND6 in the various environment.

Biography

Shan Wang is pursuing her Doctor's degree in Power Engineering and Engineering Thermophysics at Xi'an Jiaotong University. Her study focuses on the functional mechanism of the conjugative transfer system in *Pseudomonas putida* ND6 and the monitoring of conjugation in distinct environments in situ.

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Histopathological findings in surgically resected treatment-resistant epilepsy cases

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Statement of the Problem: In treatment-resistant epilepsy, different etiologies, histomorphological and immunohistochemical features, and diseases are included. Hippocampal sclerosis and focal cortical dysplasia are the most common histopathological diagnosis while tumor, vascular malformation, encephalitis, and glial scar are featured in decreasing frequency. Hippocampal sclerosis and focal cortical dysplasia are histopathologically classified according to the International League Against Epilepsy (ILAE) classifications. Molecular genetic studies in recent years have been effective in determining targeted therapies in patients who do not respond to antiepileptic drugs. mTOR pathway and immune system activation have been shown to play a role in epileptogenesis. To determine the incidence of different etiologies in the treatment-resistant epilepsy patients and find out histomorphological and immunohistochemical features and to demonstrate the relationship between the ILAE subtypes and the clinical features and try to predict the prognosis of the patients were main purposes in the neuropathological examination of our surgically resected treatment-resistant epilepsy cases.

Methodology & Theoretical Orientation: In addition to immunohistochemistry (NeuN, Neurofilament-H, CD34, GFAP, IDH-1, and Olig-2) was performed in the diagnostic process, pS6 was used to demonstrate mTOR pathway activation in FCD cases and CD3, CD8, Iba-1 antibodies were applied to demonstrate neuroinflammation in HS cases.

Findings: Statistical analysis of HS and FCD, were the most frequent histological findings, revealed a significant difference in age of seizure onset, epileptic seizure duration, surgical age, gender status, and Engel classification. pS6 expression was observed in dysmorphic neurons and balloon cells in the cases of FCD type II while lymphocyte infiltration was seen in all HS cases.

Conclusion & Significance: Significant pS6 expression in FCD type II indicates that mTOR pathway inhibitors may be involved in the treatment of epilepsy. In HS cases, no statistical significant pathological feature to predict efficacy of immunomodulating therapy in a special subgroup has been identified.

Biography

M Ozge Tepe graduated from Istanbul University, Istanbul Faculty of Medicine at the age of 24 years. She has trained in medical pathology during her residency since 2014.

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Evaluation of the antitumoral effects and mechanisms of action of novel binuclear Cu-complexes on tumorigenesis

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Cancer is one of the major causes of death across the world. Hence, the development of chemotherapeutic strategies involving novel antitumor agents has been the focus area of cancer treatment. The anticancer activities of copper complexes have been the focus of much research to discover novel anticancer agents. Current study deals with the effects of two novel binuclear copper (II) complexes with N_2O-doner tridentate ligands (R9 and R10) on cytotoxic effects on the breast (MCF-7), lung (A549) and prostate (PC3) cancer cell lines. MCF-7, A549, and PC3 cell lines were analyzed using MTT assay and Flow Cytometry intracellular ROS production assay. MCF-7, A549, and PC3 treated with R9 showed an IC50 of 1.282±0.14, 1.428±0.07 and 1.60±0.08, respectively. On the other hand, MCF-7, A549 and PC3 cell lines affected by R10 exhibited (IC_{50}=1.006±0.18, IC_{50}=1.138±0.22, IC_{50}=1.44±0.12, respectively). Flow cytometry assay for MCF-7 and A549 at three different concentrations 0.5, 1 and 2μM illustrated that cells tested with R9 and R10 presented ROS accumulation in a dose-dependent manner. In the case of testing, some of R9 and R10 concentration, the increase of ROS production was even higher than the positive control, doxorubicin. Cytotoxicity and induction of high amount of ROS may be considered R9 and R10 a potential therapeutic agent for breast, lung and prostate cancer. We will further work on these compounds to understand the exact mechanism of action of these novel complexes to pursue our investigation on their effects *in vitro* and *in vivo.*

Biography

Zeinab Ghasemishahrestani has completed her MSc in Biochemistry from Pune University in India with O grade and she is doing the PhD in UFRJ in Brazil regarding cancer research under the guidance of professors Marcos Dias Pereira and Andre Luis Souza dos Santos. She is publishing 5 papers in reputed journals.

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Survivin and caspase-3 as diagnostic and predictive biomarkers of recurrence for urinary bladder carcinoma after transurethral resection of bladder tumor

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Background: Bladder cancer even in early stage develop recurrence. Poor sensitivity of cytology and invasiveness of urethrocystoscopy have generated interest in non-invasive tools to monitor for recurrence. Caspase-3 and survivin have a central role in the regulation of apoptosis. Survivin can aid early diagnosis, determine prognosis in multiple cancer types and predict response to anti-cancer therapies. Its combination with other biomarkers as caspase-3 enhance prognostication and prediction of treatment response in Urinary Bladder Cancer or Carcinoma (UBC).

Methods: Immunohistochemical expression of survivin and caspase-3 were assessed in 44 Egyptian consecutive patients with UBC and 7 cystoscopic biopsies of cystitis as control reactive benign urothelium. Relationships between their expression, clinicopathological characteristics, diagnostic and prognostic performance were statistically analyzed.

Findings: No survivin immunoreactivity was identified in non-neoplastic bladder tissue. Expression of survivin and caspase-3 was altered in 42(95.5%) and 10(22.7%) cases, respectively. There was a statistically significant moderate positive correlation between survivin and caspase-3 expression among whole studied cases (p=.006). Expression of either survivin or caspase-3 protein individually significantly differ (p=0.000) in cancer status from control cases. Survivin was an independent predictor of UBC in multivariable analyses. Diagnostic accuracy of survivin alone was significantly better than caspase-3 alone (sensitivity 81.82% vs 68.18%, p=.027). Addition of survivin immunoreactivity to a model including caspase-3 expression improved diagnostic accuracy with a sensitivity of 93.18%. Addition of gender to the previous model improved more diagnostic accuracy with a sensitivity of 100%.

Interpretation: Survivin alone is a very promising marker and reliable indicator in UBC. Survivin and caspase-3 antigens have a cooperative effect on bladder cancer, their simultaneous evaluation augments diagnostic sensitivity.

Biography

Vivian GD Rouston was born in 1979. She obtained her MBBCh in 2004 and Master of Anatomic Pathology in 2015 from Faculty of Medicine Alexandria University. She was trained for histopathology and cytopathology at histopathology division of the Department of Pathology, St James's university hospital, the Leeds Teaching Hospitals, NHS Trust, United Kingdom. She is working as a histopathology specialist in a general hospital of the Egyptian Ministry of Health.

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HuR (ELAV1) as a potential tumor marker in gastroesophageal junction adenocarcinoma

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HuR is a master protein involved in the regulation of mRNA stability. Increased HuR expression and cytoplasmic translocation in tumors are associated with poor prognoses and altered responses to chemotherapy. HuR expression has been studied in esophageal squamous cell carcinoma, but not in gastroesophageal junction (GEJ) adenocarcinoma arising in Barrett's esophagus. To study HuR, formalin fixed paraffin embedded tissue blocks of twenty patients who underwent endoscopic mucosal resection for GEJ adenocarcinoma without pre-operative neoadjuvant therapy and five patients with Barrett's esophagus without dysplasia were retrieved upon approval of Institutional Review Board. Tissue blocks were sectioned and stained with 1:500 diluted mouse monoclonal anti-Human HuR antibody clone HuR-Rb SC-5261 (Santa Cruz, CA) and a horseradish peroxidase-conjugated secondary antibody with a Leica BOND-III automated IHC/ISH-stainer. The cytoplasmic and nuclear staining patterns of HuR were evaluated separately and scored. The intensity and cytoplasmic localization of HuR staining correlate with the neoplastic potential of the lesion. HuR staining is only detected in nuclei of benign metaplastic columnar mucosa (nuclear AIRS:2.3; cytoplasmic AIRS: 0). Barrett's epithelium shows stronger nuclear staining (AIRS:6.0) and some cytoplasmic staining (AIRS:5.2). Adenocarcinomas including poorly-differentiated adenocarcinoma and adenocarcinoma with mucinous differentiation show markedly increased HuR staining in both nuclei (AIRS:9.4) and cytoplasm (AIRS:9.4). In specimens with Barrett's epithelium and dysplasia, HuR expression appears higher in the latter. This study provides a potential novel diagnostic and differential diagnostic marker of esophageal glandular neoplasms and may also provide a novel therapeutic opportunity.

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The 14F7 Monoclonal Antibody: Past, present, and future for theragnosis in cancer

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The relevance of certain gangliosides in tumor growth and metastatic dissemination has been well documented. GM3 (NeuGc) ganglioside is particularly interesting due to its restrictive expression in normal human and chicken tissues. On the other hand, previous studies have shown that 14F7 Mab (IgG1) is a very specific anti-NeuGcGM3 ganglioside inducing cell death accompanied by cellular swelling, membrane lesion formation, and cytoskeleton activation, suggesting an oncosis-like novel phenomenon. The fact that the 14F7 Mab is able to very specific recognize in vitro and in vivo by IHC and immune gammagraphy studies the P3X63 murine myeloma cell line, the spontaneous epithelial chicken ovarian cancer and the human breast cancer that over-express the GM3 (NeuGc) ganglioside makes this Mab an important tool with anti-proliferative anti-tumor effects in vitro and in vivo animal models. A dose-escalation Phase I clinical trial is ongoing in Cuba with the humanized 14F7 Mab for studying the pharmacokinetics, toxicity and any evidence of anti-tumor effect in solid tumors over-expressing the GM3 (NeuGc). These two properties, the very specificity for recognizing tumors that over-express this ganglioside and its capability to have anti-tumor effect make this Mab an ideal drug for personalized medicine and teragnosis of cancer patients over-expressing the GM3 (NeuGc) ganglioside.

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Loss of numb in breast carcinogenesis: A paradigm for a mechanism-based selective anti-cancer stem cell therapy

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The cell fate determinant Numb is a tumor suppressor in the mammary gland whose loss in human breast cancers results in p53 inactivation and an aggressive disease course. Numb-p53 downregulation leads to aberrant mammary morphogenesis and the emergence of cancer stem cells (CSCs). Numb-deficient CSCs show unlimited self-renewal and proliferative potential, which is a function of their ability to execute unchecked self-renewing symmetric divisions. These phenotypes that can be reverted by Numb-p53 restoration in a Numb-knockout mouse model, arguing that targeting Numb-p53 dysfunction in Numb-deficient human breast cancer could represent a novel anti-CSC therapy. Using patient-derived xenografts, we have recently demonstrated that expansion of the CSC pool, due to altered self-renewing divisions, is also a distinguishing feature of naturally occurring Numb-deficient human breast cancers. In these cancers, using the inhibitor Nutlin-3 to restore p53, we corrected the defective self-renewal properties of Numb-deficient CSCs and inhibited CSC expansion, thus curbing tumorigenicity and metastasis. Remarkably, a regimen combining Nutlin-3 and chemotherapy-induced persistent tumor growth inhibition, or even regression, and prevented CSC-driven tumor relapse after removal of chemotherapy. We, therefore, provided a pre-clinical proof-of-concept that targeting Numb-p53 dysfunction results in a specific anti-CSC therapy in Numb-deficient human breast cancers. We will discuss the value of the CSC paradigm to address breast cancer heterogeneity and how functional assays based on the biology of CSCs should complement the currently used RECIST criteria for the evaluation of the efficacy of novel anti-cancer therapeutics, in the ultimate perspective of developing effective mechanism-based therapies to eradicate breast cancer.

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Pathogenic bacteria profile and antimicrobial susceptibility patterns of ear infection at Bahir Dar Regional Health Research Laboratory Center Ethiopia

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Ear infection linked with a frequent antibiotic prescription, hearing impairment, severe disability, and death is a public health threat in developing countries. However, there is a scarcity of documented data in the study area. Therefore, this study aimed at determining bacterial etiologic agents and their antimicrobial susceptibility patterns among patients of all age groups referred to Bahir Dar Regional Health Research Laboratory Center. Retrospective data recorded on culture and antimicrobial susceptibility profile were retrieved for analysis. Pus swabs from discharging ears collected and processed for aerobic bacterial culture and susceptibility testing. Of the total 368 pus swab samples processed, 296 (80.4%) were culture positive. Of which, 289 (97.6%) were bacteria and 7 (2.4%) were yeast cells. The proportion of ear infection was higher in males (92.7%) than females (65%) (P=0.014). The frequency of ear infection below 21 years of age was 65.2%. The predominant isolate was *Pseudomonas aeruginosa* (29.7%) followed by *Staphylococcus aureus* (26.3%) and *Proteus* spp. (21.9%). High level of antimicrobial resistance rates was observed for amoxicillin/clavulanic acid, ampicillin, and penicillin whereas ciprofloxacin, ceftriaxone, chloramphenicol, cotrimoxazole, gentamicin, and amikacin were found effective against the isolated bacteria. Aerobic bacterial otitis media linked with high levels of resistance against amoxicillin/clavulanic acid and ampicillin is a major health problem in the study area. Moreover, a considerable level of oxacillin-resistant S. aureus suggests the diffusion of methicillin-resistant S. aureus in the community. Therefore, treatment of otitis media in the study area needs to be guided by antibiotic susceptibility testing of isolates.

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Prevalence of antibiotic-resistant *Staphylococcus Aureus* among patients who comes to seek treatment in a hospital of Bangladesh

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Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections now become a threat and have spread worldwide. This can be very serious and are among the most frequently occurring of all antibiotic-resistant threats. The antibiotic resistance problem has been attributed to the misuse or overuse of these medications, as well as a lack of new drug development by the pharmaceutical company. In this study total 230 outdoor and indoor patients in Bangladesh Medical College and Hospital, Dhaka, Bangladesh during May 2016 to May 2017 were enrolled to detect MRSA. For this study 8 types of the biological specimen (urine, pus, blood, sputum, swab (ear/throat/high vaginal) and stool) were collected and screened for antibiotic resistance against seven (ampicillin, erythromycin, tetracycline, ciprofloxacin, gentamicin, cephalexin, and penicillin) commonly used locally available antibiotics. Among 230 total 70 samples (30.4%) were found at least resistant to one drug while drug resistance pattern was Amoxicillin, Erythromycin, Ciprofloxacin, Ceftriaxone, Cloxacillin, Cephalexin and Gentamicin.

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Automated segmentation of nucleus, cytoplasm, and background of pap-smear images using a trainable pixel level classifier

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Background: Cervical cancer ranks as the fourth most prevalent cancer affecting women worldwide and its early detection provides the opportunity to help save a life. Automated diagnosis and classification of cervical cancer from pap-smear images has become a necessity as it enables accurate, reliable and timely analysis of the condition's progress. Segmentation is a fundamental aspect of enabling successful automated pap-smear image analysis. In this paper, a potent algorithm for segmentation of the pap-smear image into the nucleus, cytoplasm, and background using pixel level information is proposed.

Methods: First, a number of pixels from the nuclei, cytoplasm, and background are extracted from five hundred images. Second, the selected pixels are trained using noise reduction, edge detection, and texture filters to produce a pixel level classifier. Third, the pixel level classifier is validated using test set and 5- fold cross validation using Fast Random Forest, Naïve Bayes, and J48 classification techniques.

Results: An extensive evaluation of the algorithm and comparison with the benchmark ground truth measurements shows promising results. Comparison of the segmented images' nucleus and cytoplasm parameters (nucleus area, longest diameter, roundness, perimeter and cytoplasm area, longest diameter, roundness, perimeter) with the ground truth segmented image feature parameters (nucleus area, longest diameter, roundness, perimeter and cytoplasm area, longest diameter, roundness, perimeter) yielded average errors of 0.94, 0.93, 0.02, 0.63, 0.96, 0.37, 0.13 and 0.96mm respectively. Validation of the proposed pixel level classifier with 5-fold cross-validation yielded a classification accuracy of 98.48%, 94.25% and 98.45% using Fast Random Forest, Naïve Bayes, and J48 classification methods respectively. Finally, validation with a test dataset yielded a classification accuracy of 98.48% and 98.98% using Fast Random Forest and J48 Classification methods respectively.

Conclusion: This paper articulates a potent approach to the segmentation of cervical cells into the nucleus, cytoplasm, and background using pixel level information. The experimental results show that the approach gives good classification and achieves a pixel classification average accuracy of 98%. The method serves as a basis for first level segmentation of pap-smear images for diagnosis and classification of cervical cancer from pap-smear images using nucleus and cytoplasm pixel level information.

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Surgical outcomes in patients with disorders of sex development in Mofid Children's Hospital, 2001-2014

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Disorders of sex development is a childhood and infantile anomaly that affect not only the somatic growth; but also leading to stress and anxiety among parents who are seeking optimal treatments. Accordingly, in this study, the surgical outcomes in patients with disorders of sex development in Mofid Children's Hospital from 2001 to 2014 were determined. In this case series study, 72 consecutive children with disorders of sex development in Mofid Children's Hospital from 2001 to 2014 were enrolled and followed in a regular manner. Data were gathered by existing medical documents and were recorded in a prepared checklist. The surgical outcomes were assessed with an interview and clinical examination after the announcement by the hospital. The success and complication rate were determined by a group of surgeons and compared according to other variables. In the current study, we have evaluated seventy-two patients: 55 (76.38%) affected by Congenital Adrenal Hyperplasia, thirteen (18.05%) by Testicular Feminization, 2 (2.7%) by Ovotesticular disorder and two cases (2.7%) by Mixed Gonadal Dysgenesis (MGD). Most common type of applied surgery was Clitoroplasty, Genitoplasty and Pull through Vaginoplasty. Fifty-nine patients (81.9%) had no surgical complications. All patients had good conditions at discharge and no mortality was registered. Three cases of testicular feminization (4.2%) who underwent pull through colovaginoplasty were married. According to our findings, surgical outcomes in cases of Disorders of Sex Development are relatively good and satisfactory. However long-term follow-up study is required to determine the final outcomes, especially for marital and sexual issues.

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Novel Slit/Robo and CXCL12/CXCR4-mediated signaling mechanisms that modulate small cell lung cancer progression and metastasis

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Small cell lung cancer (SCLC) represents 20% of lung cancers and is characterized by early dissemination, development of chemoresistance and a poor prognosis. Small cell lung cancer (SCLC) is a highly aggressive malignancy with a limited spectrum of therapeutic options. Therefore, identifying early biomarkers and targets may lead to the development of innovative therapies that will improve the survival of SCLC patients. Slit2, a secreted glycoprotein, has been shown to be suppressed in a number of cancers. Slit2 has recently emerged as an important tumor suppressor gene and acts through Roundabout Homolog1 (Robo1) receptor. Slit2/Robo1 signaling has been reported to inhibit the migration of a variety of cancer cells including non-small cell lung cancer (NSCLC). The chemokine receptor CXCR4 and its cognate chemotactic ligand CXCL12 play an important role in cell migration, cancer growth, angiogenesis, and metastasis. However, the molecular mechanism by which the Slit/Robo complex inhibits the migration of small cell lung cancer is not well defined. The aims of this study is to (1) Determine Slit2 and Robo1 expression in a wide range of pulmonary neuroendocrine carcinomas (NEC), including SCLC and in human SCLC patient samples (2) Analyze the role of Slit2 in tumor growth and metastasis in vivo using a Small cell lung cancer mouse model (3) Investigate the role of Slit2/Robo1 signaling pathway modulates the CXCL12/CXCR4-induced chemotaxis and metastasis in Small cell lung cancer.

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Association between iron deposition and neovascularization in patients with aortic valve stenosis

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Introduction: Calcific aortic stenosis (AS) is characterized by calcification, restricted leaflet motion, and reduction in valve area. It has been shown that neovessels, intraleaflet hemorrhage, and iron deposition may be involved in the pathophysiology of the disease. We sought to evaluate the association between aortic valve intraleaflet neovascularization and iron deposition in patients with AS using histologic techniques.

Methods: Aortic valve leaflets of 10 consenting patients with AS were extracted during surgical aortic valve replacement, fixed, embedded and sectioned. Presence of microvessels, macrophages, and iron was assessed with standard immunohistochemical staining with CD34, CD68 and iron respectively. Histological analysis was performed using Leica software. For each stain, the areas of CD34, CD68, and iron positive pixels were calculated. To assess linear dependence between variables, the Pearson's correlation coefficient for normally distributed or Spearman's rank correlation coefficient for non-normally distributed variables was calculated. A value of $p<0.05$ was considered statistically significant.

Results: The demographic characteristics, risk factors, and echocardiography of study patients. There was a positive correlation between detection of cd34 cells and iron (r=0.7, P=0.02). Detection of CD34 cells was also positively correlated with the peak transvalvular gradient (0.41, p=0.5), and negatively associated with valve area (r=-0.14, p=0.05).

Conclusion: Our results suggest that iron deposition occurs in association with neovascularization. These preliminary findings support the hypothesis that angiogenesis may promote intraleaflet inflammation by allowing iron deposition.

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Novel Angiotensin Receptor Blocker, Azilsartan induces oxidative stress and NFkB-mediated apoptosis in Hepatocellular Carcinoma cell line HepG2

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Overexpression of renin-angiotensin system (RAS) and nuclear factor-kappaB (NF-kB) has a key role in various cancers. Blockade of RAS and NF-kB pathway has been suggested to reduce cancer cell proliferation. This study aimed to investigate the role of angiotensin II and NF-kB pathway in liver hepatocellular carcinoma cell line (HepG2) proliferation by using azilsartan (as a novel Ag II antagonist) and Bay11-7082 (as NF-kB inhibitor). HepG2 cells were treated with different concentrations of azilsartan and Bay11-7082. Cytotoxicity was determined after 24, 48, and 72 h by MTT assay. Reactive oxygen spices (ROS) generation and cytochrome c release were measured following azilsartan and Bay11-7082 treatment. Apoptosis was analyzed qualitatively by DAPI staining and quantitatively through flow cytometry methodologies and Bax and Bcl-2 mRNA and protein levels were assessed by real-time PCR and ELISA methods, respectively. The cytotoxic effects of different concentration of azilsartan and Bay11-7082 on HepG2 cells were observed as a reduction in cell viability, ROS formation, cytochrome c release, and apoptosis induction. These effects were found to correlate with a shift in Bax level and a downward trend in the expression of Bcl-2. These findings suggest that azilsartan and Bay11-7082 in combination or alone have strong potential for development as an agent for prevention against liver cancer after further studies.

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Prevalence and antibiogram profiles of *Escherichia Coli* O157:H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa

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Escherichia coli O157:H7 is one of the most imperious foodborne pathogens predisposed for a number of mortalities worldwide. To investigate the occurrence and antibiotics susceptibility of *Escherichia coli (E. coli)* from three selected commercial dairy farms in the Amathole District Municipality, Eastern Cape Province, South Africa, raw milk samples were collected from bulk storage tanks and swab samples from milking machines, cattle udder(s) and workers hands were also collected on a six-month sampling regime between June and November 2014. A standard culture-based method was used for the enumeration and isolation of *E. coli* O157:H7 using sorbitol MacConkey agar (supplemented with cefixime (50µg/L) and potassium tellurite (25mg/L). A serological confirmation of the presumptive *E. coli* O157:H7 isolates was conducted using the O157 latex agglutination test kit. A total of 252 *E. coli* O157:H7 isolates were further subjected to PCR detection of rfbE O157 and fliCH7 genes of which 27 (11%) of the isolates were confirmed positive *E. coli* O157:H7. Our finding reveals that of the 27 *E. coli* O157:H7 isolates from the dairy farms, the rate of resistance against penicillin was 85% and resistance against the other antibiotics follow the order: tetracycline (81%), erythromycin (70%), streptomycin (52%) and chloramphenicol (45%). We conclude that the dairy farms are potential reservoirs of *E. coli* O157:H7 serotype with multiple antibiotic resistance and consequently a concern to public and environmental health.

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Molecular basis and mechanism of resistance to Ciprofloxacin by *Staphylococcus Aureus* strains isolated from pregnant women

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Staphylococcus aureus can cause worrisome infections especially for the immune compromised pregnant woman and her fetus but worse problems are posed by the drug-resistant strains. This study was carried out to determine the molecular basis and mechanism of resistance of *Staphylococcus aureus* to ciprofloxacin; a quinolone with broad-spectrum antimicrobial activities. Five known ciprofloxacin-resistant *Staphylococcus aureus* strains isolated from pregnant women attending antenatal clinics in Imo State Nigeria were used for this study. Their antibiotic resistance profiles were confirmed using disc diffusion method. Minimum inhibitory concentration (MIC) of ciprofloxacin on test isolates was also obtained using standard microbiological tests. This was followed by molecular studies which involved; Genomic DNA extraction, polymerase chain reaction, gel electrophoresis, and gene sequencing. Analysis of the sequences obtained was done using the clcbio main workbench software to obtain their statistics, basic alignment, and phylogeny. Results revealed resistance to ciprofloxacin to be genetic with all the isolates harbouring the quinolone resistance determinant region, (QRDR) found on Gyr A and Par C genes. BLAST results with related genes in the gene bank showed mutations at the quinolone target site suggestive of modification of the target site as a mechanism of resistance observed. Phylogenetic analysis revealed that the genes studied were from one ancestor hence possible horizontal transfer of resistance genetic materials among the isolates. The public health importance of this cannot be overemphasized.

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Prevalence and rapid diagnosis of acute bacterial meningitis in children in Bangladesh

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An attempt was made to analyze the cerebrospinal fluid (CSF) profile and to isolate and identify aetiological agents from the specimens from children with suspected acute bacterial meningitis. Among total 79 samples, 65 (82.3%) were crystal clear, 9 (11.4%) were moderately turbid, 2 (2.5%) highly turbid and remaining 3 (3.8%) were high blood mixed. The total cell (leucocyte) count of the CSF was proportionate to the turbidity. In case of crystal clear CSF's, total leucocyte counts were normally ranging from 0 to 700 per mm3 with predominant lymphocytes. Moderately turbid fluid showed 200 to 2,000 cells per mm3 and highly turbid fluid and highly blood mixed showed more than 40,000 cells per mm3. In the later cases, differential counts demonstrated polymorphonuclear predominancy. In 65 cases whose CSF were crystal clear, total protein and sugar concentration ranged from 20 to 400mg/dl and 20 to 180mg/dl respectively. In turbid CSF's, total protein and sugar concentration varied from 70 to 500mg/dl and 10 to 200mg/dl respectively, while in the highly turbid CSF's, they ranged from 50 to 800mg/dl and 40 to 140mg/dl respectively. Among total 79 CSF samples, Pandey's tests were positive for 16.9% and negative for 9.2% in cases of the crystal clear. In case of moderately turbid and highly turbid CSF's, Pandey's test was positive for 88.9% and 100% cases respective. C-Reactive protein (CRP) were positive (>12mg/dl) for 3 (3.79%) samples. A total of 79 CSF was culture. There were 5 culture positive cases, which included *Escherichia coli* (20%), *Haemophilus influenzae* (20%) and *Streptococcus pneumoniae* (60%). Using the latex agglutination test, the detection rate was higher than that of culture. Most of meningitis positive cases showed an increased total cell counts as well as proteins concentration and decreased serum sugar concentrations. High resistant rate to cotrimoxazole was observed among the invasive isolates. On the other hand, none of these invasive strains showed resistant to ceftriaxone.

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HMG2 and PLAG1 protein expression in Pleomorphic Adenoma Tumorigenesis and its recurrence and in the progression to Carcinoma ex-Pleomorphic Adenoma

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The Pleomorphic Adenoma (PA) is the most common neoplasm of salivary glands. Recurrences of APs are common and increase the probability of the malignant transformation giving rise to the Carcinoma Ex Pleomorphic Adenoma (CXPA), which, although rare, is an aggressive tumor with frequent metastasis and death related to the disease. As previously reported in the literature, the Pleomorphic Adenoma Gene 1 (PLAG1) and High Mobility Group AT-hook 2 (HMGA2) genes are associated with the onset and progression of PAs and CXPAs. HMGA2 plays a role in the architectural transcription factor, modulating the three-dimensional conformation of the DNA and consequently modulating the expression of several genes. The PLAG1 gene is involved in cell proliferation through the control of various target genes. In normal tissues, its activity is high during embryonic and fetal development, but in adult life, however, its participation is low or absent. The protein expression of PLAG1 and HMGA2 in 38 cases of PA, 36 cases of Recurrent PA (RPA) and 41 cases of CXAP was analyzed. The histological subtype and degree of tumor progression were considered. A significant association of PLAG1 with PAs was found (89.5%), while the HMGA2 gene protein was presented with a relevant association with the malignant counterpart of the disease (48.78%). A higher prevalence of HMGA2 protein expression in high grade and aggressive tumors considering the histological subtype and degree of tumor progression was observed. PLAG1 protein expression was lower when PA underwent malignant transformation, possibly due to other pathway activation and different clone cells. In addition, PLAG1 protein expression was present mainly in low-grade carcinomas and in cases with the early phase of invasion probably due to its property of regulation of oncogene-induced cell senescence. In CXPA, PLAG1 expression was mostly associated with myoepithelial differentiation. This way, the loss of PLAG1 protein expression can be considered a hallmark of CXPA carcinogenesis, mainly when there is only epithelial differentiation. Our study showed that these genes are promising targets for more effective therapies and consequently lower morbidity due to these neoplasms.

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Co-delivery of anti-cancer drugs by combination therapy

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Among many cancer therapies, chemotherapy and photodynamic therapy (PDT) have been considered in this essay. For the enhancement of the drug delivery, the use of the up-conversion material is taken into account. Under near-infrared (NIR) excitation, up-conversion emits ultraviolet light. in the traditional PDT, injecting the photosensitizer (PS), as a drug, then using illumination source like laser, light emitting diodes, arcing lamps and laser in order to active PS. Using up-conversion can help the drug to penetrate in more depth of the tumor tissue, compared to the traditional PDT, and improve the efficiency of the drug and finally the cancerous cell death. Additionally, we could assemble a core-shell nanoparticle to improve the chemotherapy as well as PDT. In regard to this, we could conjugate doxorubicin (DOX) in the shell and then (PS) in the core in order to deliver two anti-cancer. Then, the nanoparticle is PEGylated to overcome the dilemma of "protein corona". Also, using folic acid (FA) for the cancerous cell receptor, as a ligand, in the endocytosis-mediated process, we could guarantee the targeted therapy.

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Delving KS-01 as a novel therapeutic strategy in treating breast cancer

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Cancer cells have an increased need for cholesterol, which is required for cell membrane integrity. Cholesterol accumulation has been described in various malignancies including breast cancer. Cholesterol has also been known to be the precursor of estrogen and vitamin D, both of which play a key role in the histology of breast cancer. Thus, depleting the cholesterol levels in cancer cells is a proposed innovative strategy to treat cancer. Therefore, novel cholesterol-depleting compounds are currently being investigated. KS-01 is a cyclic amylose oligomer composed of glucose units. It solubilizes the cholesterol and is proven to be toxicologically benign in humans. This led us to hypothesize that it might deplete cholesterol from cancer cells and may prove to be a clinically useful compound. Our work provides preliminary experimental evidences to support this hypothesis. We identified the potency of KS-01 in vitro against two breast cancer cell lines: MCF-7 (Estrogen positive, ER+), MDA-MB-231 (Estrogen negative, ER-) and compared the results against two normal cell lines: MRC-5 (Normal Human Lung Fibroblasts) and HEK-293 (Normal human embryonic kidney cells) using cytotoxic, apoptosis and cholesterol based assays. KS-01 treatment reduced intracellular cholesterol resulting in significant breast cancer cell growth inhibition through apoptosis. The results hold true for both ER+ and ER-. These data suggest that KS-01 can prevent cholesterol accumulation in breast cancer cells and is a promising new anticancer agent.

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Investigation of itaconate metabolism in *Cupriavidus Necator* H16

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Recent challenges of pollution and climate change in our environment stems from the over-dependence on fossil fuel through the extraction, processing, and exploitation for petrochemical-based products. This has caused severe havoc to the environment and its natural habitats, leading to deaths and displacements into unfavorable conditions. Researchers in the US Department of Energy (DoE) in 2004 identified itaconate, one of the twelve attractive platform chemicals, as a potential chemical suitable for bio-based industrial products using biological routes. Previous research has also shown that itaconate has the potential to replace petroleum-based products such as petrochemical-based acrylic and methacrylic acid; and detergents, surface active agents and biosynthesized plastics for industrial applications with bio-based products. This can be achieved through biological or chemical conversions and be subsequently converted into several high-value bio-based chemicals and materials from biomass. Research also discovered that itaconate is naturally produced by microorganisms such as *Candida* sp., *Ustilago madis* and *Aspergillus terreus* although many microorganisms have been genetically engineered for the biosynthesis of itaconate. It is, therefore, necessary for the current generation to identify various sustainable and cleaner processes for chemical, fuel and energy production. HPLC was used to estimate the concentration of itaconate consumed. The purpose of this research was to identify the genes involved in itaconate metabolism and abolish its metabolism. To investigate itaconate metabolism on host organism *Cupriavidus necator* H16, the growth of mutants was observed using itaconate as a sole carbon source. Single, double and triple knock-outs of ict genes involved in itaconate conversion to itaconyl-CoA (itaconate-CoA transferase activity) were generated. Growth and itaconate consumption assays were performed establishing that only H16_RS22140 gene is clearly involved in itaconate metabolism. This study revealed that other genes can be involved in itaconate degradation and therefore further research to investigate the function of these genes is required.

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Incorporation of *Vitreoscilla* hemoglobin gene mitigates biofilm formation in *Bacillus* Subtilis DK1042

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Biofilm formation is often considered as a stress combating strategy adopted by bacteria in response to a variety of cellular and environmental signals. Impaired respiration triggers biofilm formation in *B. subtilis*. *Vitreoscilla* hemoglobin (VHb) is known to supply oxygen to respiratory chain and hence improves aerobic growth and bioproduct synthesis of a variety of bacteria including *Bacillus spp*. Although VHb improves respiration, very little efforts have been made in elucidating its effect on biofilm formation. *B. subtilis* DK1042 was genetically modified to develop two integrants NRM1113 and NRM1114 containing vgb-gfp operon under 2 and 5 copies of P43 promoters, respectively, at an amyE locus by double-crossover events. Effect of VHb on biofilm formation by integrants and wild-type (WT) was assessed on both solid and pellicle biofilm in lysogeny broth (LB) and LB supplemented with 1% glycerol and 0.1mM manganese (LBGM). Here, we report that genomic integration of vgb gene in *B. subtilis* DK1042 mitigates biofilm formation and associated sporulation under different conditions. It also decreases the sporulation associated brown pigment production in minimal medium in shake flask cultures. These findings suggest that VHb mediated prolonged vegetative state may augment the production of desired bioproducts by host Bacillus spp. Reduced biofilm forming phenotype in LBGM medium and hyperosmotic conditions indicates that VHb has a profound impact on entire regulatory network governing biofilm formation. Use of VHb harboring *Bacillus* biofertilizers will have a tremendous advantage during their sessile lifestyle in rhizosphere that may enhance their performance as Plant Growth Promoting *Rhizobacteria* (PGPR) and Rhizoremedial agents.

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Gas-producing *Vibrio Cholerae*: A case report of gastroenteritis with acute kidney injury

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We herein report the first case of gas producing *Vibrio cholerae* gastroenteritis with an acute kidney injury. A 30-year-old female presented to the emergency department with complaints of about 10 episodes of watery diarrhea, 4 episodes of vomiting and elevated serum urea/creatinine levels. Although the bacteria were first misidentified as *V. furnissii* by gas production on triple sugar iron agar, it was later confirmed as *Vibrio cholerae* by 16S rRNA gene sequencing and specific PCR. The treatment regimen was followed as for *Vibrio* species with Intravenous fluids, Ciprofloxacin and Doxycycline. The patient recovered without relapse.

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H3N2 influenza vaccine rates and other protective behaviours amongst college students

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Influenza infections can results in seasonal outbreaks and epidemics in the USA. The 2014-2015 influenza outbreak was attributed to the H3N2 influenza A strain. This outbreak was partly attributed to the mismatch between the causative H3N2 influenza A strain and the annual influenza vaccine. The aim of this study was to determine if the mismatch between the causative influenza strain and the vaccine impacted vaccine rates or other protective health behaviour's amongst college students. In this study, an online survey was used to determine the rate the influenza vaccination rates and any changes in student hygienic behaviours during the 2014-2015 influenza season amongst college students. Survey responses were collected from Jan. 15, 2015 to Feb. 15, 2015, and elicited 265 responses from undergraduate students. The total vaccine rate among respondents was 23%, but compared to the previous year (2013-2014) the overall vaccination rate among respondents decreased by 10%. Regardless of vaccination, 53% of total respondents reported a 'slight change' or 'more' in the protective health behaviour of hand-washing. The influenza vaccination rate amongst college students is within the range of the national CDC vaccination rate of 31% for this age group. The decrease in vaccination rates from 2013-2014 to 2014-2015 was consistent with the mismatch between the influenza strain and vaccine targets. Beyond vaccination, protection against influenza also involves enhanced personal and hand-hygiene behaviours. Such behaviours are very important on a college campus due to close living conditions and other social and casual behaviours.

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